

# Transition Metal-based Anticancer Drugs Targeting Nucleic Acids: A Tandem Mass Spectrometric Investigation

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**Abstract:** The search for effective drugs against cisplatin-resistant tumors resulted in a large number of organometallic compounds that are evaluated for their antiproliferative activity. Among the most promising candidates are bent metallocenes based on various transition metal ions and ligands. The elucidation of structural features and the characterization of the interaction of a drug candidate with its target require accurate and sensitive analytical tools. Tandem mass spectrometry is applied to the investigation of the adduct sites and binding patterns of metallodrugs bound to single-stranded oligonucleotides and higher-order nucleic acids. Results reveal the binding specificities of the different metallodrugs and demonstrate the influence they exert on the dissociation pathways of the adducts in the gas-phase.

**Keywords:** Anticancer drugs · Cisplatin · Metallocenes · Nucleic acids · Tandem mass spectrometry

## Introduction

Research in the life sciences has been strongly accelerated over the past two decades by the availability of mass spectrometry-based techniques for the analysis of peptide, protein, and lipid biomarkers, as well as drugs and their metabolites. Owing to its high selectivity and sensitivity, tandem mass spectrometry has evolved into one of the most powerful tools for the elucidation of molecular structures, the sequencing of biopolymers, and the comprehensive quantitation of organic compounds in complex matrices. The characterization of complex mixtures can be approached by a combination of chromatographic separation, soft ionization, and high-resolution mass analysis. Furthermore, the ability of tandem mass spectrometry (MS/MS) to select and activate precursor ions in the gas-phase makes the method inherently well suited for the analysis of heterogeneous samples. In contrast to the rapid expansion of mass spectrometry-

based proteomics and diagnostics, the characterization of nucleic acids remains a niche application. Nevertheless, the identification and quantitation of nucleic acid modifications, the sequencing of highly modified therapeutic oligonucleotides, and the elucidation of drug-nucleic acid interactions represent key applications that benefit from the strengths of tandem mass spectrometry.

## Sequencing of Nucleic Acids by Tandem Mass Spectrometry

When seen from a distance, the tandem mass spectrometric sequencing of any biopolymer might essentially follow the same principle. Analyte ions of interest are isolated in the gas-phase and their internal energy is increased, commonly by collision with an inert gas in a process referred to as collision-induced dissociation (CID). As a result of this activation, covalent bonds are cleaved and one or more series of backbone fragments are generated, which provide the desired sequence information. However, due to unique structural features, peptides and oligonucleotides exhibit completely different gas-phase behaviors. While peptides predominantly dissociate by scission of the amide bond that links two adjoining amino acids, the structure dependence of oligonucleotide fragmentation is much more pronounced and more often than not, several dissociation events coincide. The backbone cleavage of deoxyribonucleic acids (DNA) is always accompanied by the loss of a nucleobase, as this represents the initial step of the dissociation mechanism

eventually resulting in the cleavage of the so-called (a-B)- and w-fragment ions (Fig. 1).<sup>[1]</sup> In contrast, the cleavage of the 5'-P-O phosphoester bond in ribonucleic acids (RNA) is independent of nucleobase loss and results in the c- and y-fragment ions.<sup>[2,3]</sup> Oligonucleotides administered for therapeutic purposes (*e.g.* for antisense applications) commonly comprise structural modifications of the backbone, the sugar moiety, or the nucleobases in order to improve their biostability and to tailor their interaction with the complementary nucleic acid targets. Such modifications influence the dissociation pathways of the oligonucleotides and, consequently, affect the resulting product ion spectra.<sup>[4–6]</sup> In any case, charge-induced nucleobase loss, the occurrence of internal fragments due to secondary dissociation events, and the presence of ubiquitous alkali ion adducts further complicate the mass spectral data.

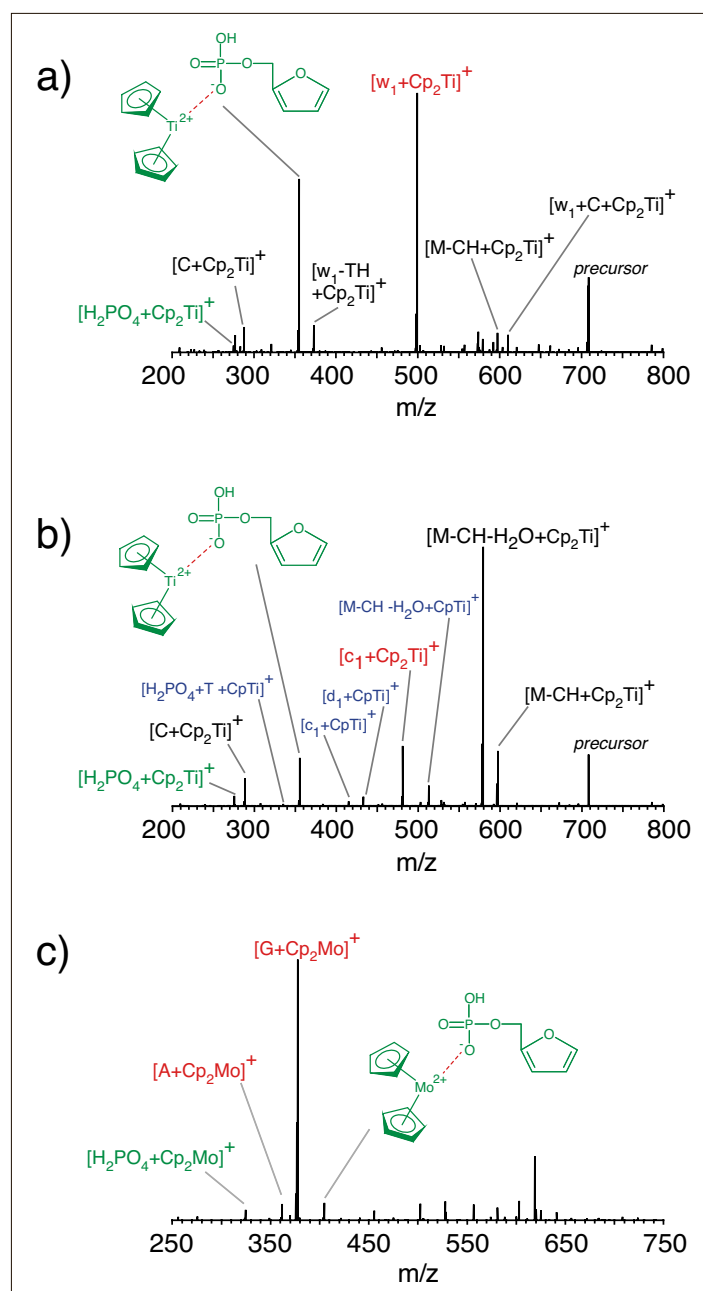
## Software-supported Spectra Interpretation

The great diversity of possible dissociation products turns the elucidation of nucleotide sequences and their structural modifications into a very time-consuming and error-prone process. Spectra interpretation is greatly facilitated by the software tool OMA & OPA (oligonucleotide mass assembler & oligonucleotide peak analyzer) developed in our department.<sup>[7]</sup> The Java-based software calculates the masses of all theoretically possible fragments of a given single-stranded oligonucleotide or duplex

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Fig. 2. Product ion spectra generated by collisional activation of the adducts of a) d(CpT) and b) d(TpC) with titanocene and c) d(ApG) with molybdenocene. In the applied nomenclature, G corresponds to a negatively charged guanine, whereas GH indicates a neutral nucleobase.



guanine nucleobase is much stronger than the interaction with the adjacent adenine, as reflected by the low peak intensity of  $[A+Cp_2Mo]^+$ .

### Higher-order Nucleic Acids as Drug Targets

Due to their important roles in cellular processes, higher-order structures, such as duplex- and quadruplex-DNA, represent potential drug targets as well. Despite the non-covalent binding characteristics of these assemblies, they do not necessarily separate upon electrospray ionization and collisional activation. Experiments on non-platinated and platinated quadruplexes revealed the formation of truncated

structures due to the cleavage of covalent bonds in the oligonucleotide backbone without disrupting all non-covalent interactions between the nucleobases.<sup>[13,18]</sup> This observation was somewhat unexpected, because low-energy CID is considered as a slow-heating ergodic process, which redistributes the added internal energy among the vibrational modes of the precursor ion prior to fragmentation and, therefore, was expected to result in the disruption of the non-covalent nucleobase interactions. In some cases, the detection of adducted and unadducted fragment ions allowed the localization of the adduct sites. Nevertheless, experimental alternatives are necessary when strand separation impedes precise characterization of the binding pattern.

### Alternative Ion Activation Techniques

The various techniques used for ion activation in the gas-phase do not only differ in the amount of transferred energy, but also in the time frame required for the transfer of sufficient energy and in the mechanisms responsible for the dissociation of the precursor ions. The considerable long time frame required for low-energy collisional activation (up to 100 ms) enables the redistribution of the added internal energy over the many degrees of freedom of the ions prior to fragmentation. This results in an ergodic dissociation behavior, slows down the reaction rate of covalent bond cleavage in large ions, and promotes the cleavage of non-covalent interactions, thus, impeding the investigation of higher-order structural features.

Alternative activation techniques that prevent energy randomization are applied to overcome this drawback. Among them are radical-based methods such as electron capture dissociation (ECD) and electron transfer dissociation (ETD). Transfer of electrons to a precursor ion population stored in a trapping instrument triggers odd-electron dissociation channels that provide sequence and structural information orthogonal to the data obtained by CID. The effect of various activation techniques on the types of generated fragment ions has been demonstrated for oligonucleotide-cisplatin adducts.<sup>[12]</sup> ETD was found to primarily result in charge reduction of the precursor ions, but backbone fragments could be generated by additional collisional activation of the charge-reduced precursor ions. The transfer of an electron to nucleic acids is highly nucleobase-specific with a high preference for abstraction by a cytosine nucleobase.<sup>[17,18]</sup> Our recent ETD experiments on metallocene-DNA adducts of different charge states provided deeper insight into the radical-driven dissociation. Electron transfer to the doubly charged hexamer adduct resulted in charge reduction only. Interestingly, electron transfer to the triply charged precursor ion led to the doubly reduced adduct. The first electron was transferred to the metal center and the second one abstracted by the cytosine nucleobase, where it induced backbone cleavage (Fig. 3). The extent of electron transfer to the adduct was found to depend on the coordination center, which is likely to affect the dissociation mechanism. Elucidation of this mechanism represents the initial step on the way to extracting orthogonal structure information on higher-order nucleic acid assemblies and identifying the binding characteristics of metallodrugs.

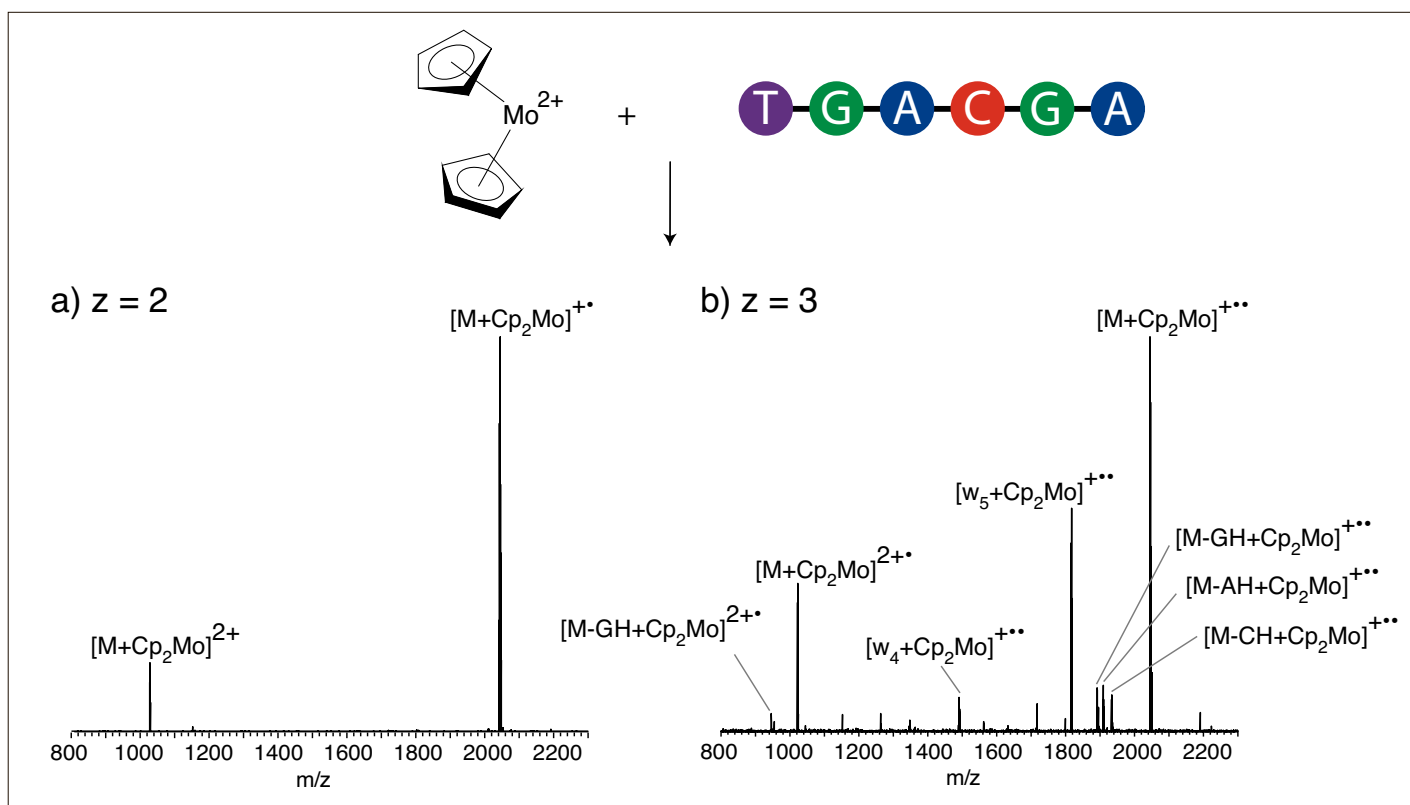


Fig. 3. Electron transfer dissociation of doubly and triply charged molybdenocene-DNA adducts.

## Conclusion

The combination of accurate mass high-resolution mass spectrometry with multiple ion activation techniques forms a powerful toolkit for the investigation of biomolecule-drug interactions. Among the analytical techniques applied in the life sciences, tandem mass spectrometry excels with high sensitivity paired with the ability to isolate and activate precursor ions of interest in the gas-phase without the need for high sample purity. Knowledge on the dissociation behavior of the compound class of interest is an essential prerequisite for the reliable interpretation of the analytical data and, consequently, for the proper characterization of the biomolecule-drug adducts.

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